Food Chemistry 192 (2016) 837–841

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Preliminary results of mercury levels in raw and cooked seafood and their public health impact

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ARTICLE INFO

Article history:
Received 21 June 2014
Received in revised form 9 July 2015
Accepted 20 July 2015
Available online 21 July 2015

Keywords:
Mercury
Bivalves
Fish
Crustaceans
Cooking effect
Todos os Santos Bay

ABSTRACT

Mercury is toxic for human health and one of the main routes of exposure is through consumption of contaminated fish and shellfish. The objective of this work was to assess the possible mercury contamination of bivalves (*Anomalocardia brasiliana*, *Lucina pectinata*, *Callinectes sapidus*), crustacean (*C. sapidus*) and fish (*Bagre marinus* and *Diapterus rhombeus*) collected on Salinas da Margarida, BA (Brazil), a region which carciniculture, fishing and shellfish extraction are the most important economic activities. The effect of cooking on Hg concentration in the samples was also studied. The results showed that Hg concentration was generally higher in the cooked samples than in raw samples. This increase can be related to the effect of Hg pre-concentration, formation of complexes involving mercury species and sulfhydryl groups present in tissues and/or loss of water and fat. The highest concentrations were found in *B. marinus* samples ranging 837.0–1585.3 μg kg^{-1}, which exceeded those recommended by Brazilian Health Surveillance Agency (ANVISA). In addition, Hg values found in the other samples also suggest the monitoring of the Hg concentrations in seafood consumed from the region.

1. Introduction

Mercury (Hg) is an environmental pollutant due to its high toxicity, even at low concentrations, and its capacity to bioaccumulate in organisms and biomagnify in the food chain (US EPA, 1997, 2003; Porto, Araujo, & Feldberga, 2005; UNEP, 2013). In the environment, the inorganic forms (Hg^0 and Hg(II)) are easily transformed in organic species (Bisinoti & Jardim, 2003; Poulin & Gibb, 2008; Voegborlo & Akagi, 2007). Among the main transformations is the formation of methylmercury (MeHg) (Blum, Popp, Drazen, Choy, & Johnson, 2013). This chemical species enters into the aquatic food chain and reaches its maximum concentration in the tissues of fish at the top of this chain, and MeHg accounts for 80% to 95% of the total mercury (Afonso et al., 2008; Blum et al., 2013; EFSA, 2012). The its most serious effects in humans are damages in the nervous system (FAO/WHO, 2004; Hightower & Moore, 2003).

Due to the capable of Hg accumulating along the trophic chain, especially in aquatic environments, human exposure to mercury occurs mainly through the consumption of fish and seafood in which a significant concentration of mercury is present (Hightower & Moore, 2003; US EPA, 2009). Additionally, an increase of mercury concentrations has been observed around the world in several fish species along the last decades, some with levels exceeding human toxicological thresholds (US FDA, 2013; US EPA, 1997, 2003, 2004, 2009). The World Health Organization (WHO) assumes that foods with mercury concentrations of 0.5 mg kg^{-1} (wet weight) or more are inadequate for human consumption (WHO, 1996). In Brazil, the maximum limits of mercury are 0.5 mg kg^{-1} and 1.0 mg kg^{-1} for non-carnivorous and predatory fish, respectively (ANVISA, 1998, 2013).

Recent studies evaluated the Hg concentrations and their species after different culinary treatments in fish, and according to the authors no significant conversion of Hg species or variation in the total Hg concentrations were observed by this treatments (Schimidt et al., 2015). However, there are still many controversies about the influence of cooking in the Hg concentrations (Amyot & Ouédraogo, 2011; Kalogeropoulos et al., 2012; Perello, Martí-Cid, Llobet, & Domingo, 2008; Schimidt et al., 2015).

Salinas da Margarida is a Brazilian municipality, which belongs to the state of Bahia and it is known as one of the major Brazilian producers and distributors of shrimp. In addition to carciniculture, the main economic activities are fishing and shellfish extraction (IBGE – Instituto Brasileiro de Geografia e Estatística., 2013). Around the year of 2009, an oil rig was implanted in São Roque do Paraguaçu, a locality near Salinas da Margarida, and it is known that Hg, as well as some other trace elements, is present in all geological hydrocarbons (Sofowote et al., 2011).

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http://dx.doi.org/10.1016/j.foodchem.2015.07.081
0308-8146/

The present work is the first one to examine the Hg concentrations, in cooked and raw samples of *Anomalocardia brasiliana*, *Lucina pectinata*, *Callinectes sapidus*, *Bagre marinus* and *Diapterus rhombeus* from Salinas da Margarida, Todos os Santos Bay (TSB), in order to evaluate the possible Hg contamination in the commercialized and consumed seafood after the implantation of the oil rig.

2. Materials and methods

2.1. Sampling

Samples of two bivalve mollusks (*L. pectinata*, *A. brasiliana*), one crustacean (*C. sapidus*) and two fish (*D. rhombeus* and *B. marinus*) were collected during two periods, in May and June of 2013, on Salinas da Margarida. Two samples of each organism were collected in both sampling periods, totalizing 10 samples. Approximately 1.0 kg of *A. brasiliana*, *L. pectinata* and *C. sapidus* were collected in surface sediment (up to 20 cm deep) during low tide. Ten *D. rhombeus*, up to 150 g each, were caught with cast nets at depths up to 10 m. Three *B. marinus*, approximately 1.0 kg each, were caught by hooks and fishing line at depths between 20 and 50 m. Immediately after being caught, fish were slaughtered by severing the spinal cord, in accordance with Brazilian laws (BRASIL, 2000). The fish samples were identified, labeled in separate polyethylene bags, transferred in ice boxes, and transported to the laboratory. Then muscle of samples was removed and homogenized.

2.2. Reagents

Analytical grade reagents were utilized (Merck, Darmstadt, Germany), a standard reference solution of 1.000 mg L^{-1} (Merck, Darmstadt, Germany) was used to build the Hg analytical curve, and purified water obtained from a Milli-Q system (Millipore, Billerica, MA, USA) was used to prepare all of the aqueous solutions.

2.3. Sample preparation

All samples were separated in two groups for the evaluation of the mercury levels before and after cooking to observe if there are some variations in the Hg concentrations in the cooked samples. Therefore, one part of the sample was only dried in an oven with air circulation at 60 °C for 48 h, and it was then ground in an impact mill. The other part was placed in a beaker with approximately 1 L of ultrapure water and heated on a hot plate for 30 min at a temperature of 80 °C. After cooking, the samples were dried and ground under the same conditions as the raw samples. It is noteworthy that the analysis was performed on entire crustacean, the shells were removed from the bivalve mollusks before analysis, and only the muscles of the fish were analyzed.

2.4. Apparatus

The analyses were carried out with a Direct Mercury Analyzer® (DMA-80, Milestone Srl, Italy), according to adapted method US EPA (2007). This equipment typically contains an automatic sampler, a quartz furnace, a cobalt-manganese oxide catalyst, a gold-coated sand amalgamator and an atomic absorption detection cell with three different path lengths (165, 120 and 4 mm). The sample is introduced into the quartz furnace, where it is heated up to 200 °C (drying temperature) for 60 s and 650 °C (ashing temperature, maximum temperature allowed by the software of the equipment) for 105 s, which allows Hg volatilization and reduction. Air (99.99%) was used as the combustion and carrier gases (Melendez-Perez & Fostier, 2013).

2.5. Optimization tests

It was first assessed whether the measurement of the mercury concentration could be significantly affected by the sample amount analyzed. Some collected samples (bivalves, fish and crustaceans) were used to assess the effect of mass variation between 5 and 100 mg.

2.6. Validation of the analytical method

The limits of detection (LOD) and quantitation (LOQ) were determined as 3 and 10 times the standard deviation of the residuals from the linear regression, respectively, divided by the angular coefficient value of the linear equation (Miller & Miller, 2000). For each type of sample (fish, crustacean and bivalves), the precision was assessed by the relative standard deviation from ten analytical replicates of one sample. The accuracy was checked by the addition and recovery of two concentrations of Hg(II), 1 ng and 10 ng, and by the analysis of the following three certified reference materials: DOLT-4 (Dogfisher liver), NIST 1566th (Oyster tissue) and MURST ISS-A2 (Antarctic krill). For the evaluation of matrix effects, calibration curves were constructed using samples spiked with known amounts of Hg(II) standard solution in each matrix. The slope of each curve was compared with the slope of the curve constructed in acidic solution.

2.7. Statistical analysis

Mercury contents were calculated on a fresh weight (fw) basis. Data were expressed as the mean ± standard deviation, and the differences between the mercury contents of the raw and cooked samples in both sampling periods were assessed using a two-tailed Student's *t*-test for $n = 3$ with a 95% confidence level.

3. Results and discussion

3.1. Effect of the sample mass

Fig. 1 shows the effect of the sample mass on the determined Hg concentrations for bivalves, fish and crustaceans. No significant difference was observed at the confidence level of 95% between masses of 10 and 50 mg for the crustacean samples, 5 and 30 mg for the bivalves and 20 and 60 mg for the fish. Thus, it was weighed 10 mg of bivalves and 30 mg of fish and crustacean.

3.2. Method validation

The limits of detection and quantification were 0.021 ng Hg and 0.072 ng Hg, respectively. The linear range was from 0.1 ng to 25 ng Hg. Accuracy was determined by the analysis of reference materials and addition and recovery tests. The results obtained were in good agreement with the certified values, with recoveries for the reference materials ranging from 91% to 107% and from 85% to 89% for the addition and recovery tests (Table 1). The precision (RSD) was generally less than 4% (Table 1).

3.3. Mercury concentrations in fish, bivalves and crustacean

Mercury concentrations were expressed in micrograms per kilogram wet weight taking into account the humidity which was of 77% for the raw fish and 80% for the raw bivalves and crustacean. The concentrations in the raw samples ranged 837.0–1585.3 μg kg^{-1} for *B. marinus*, 53.0–212.0 μg kg^{-1} for *D. rhombeus*, 365.0–725.0 μg kg^{-1} for *L. pectinata*, 124.0–203.0 μg kg^{-1} for *A. brasiliana* and 83.0–149.0 μg kg^{-1} for *C. sapidus* (Fig. 2).

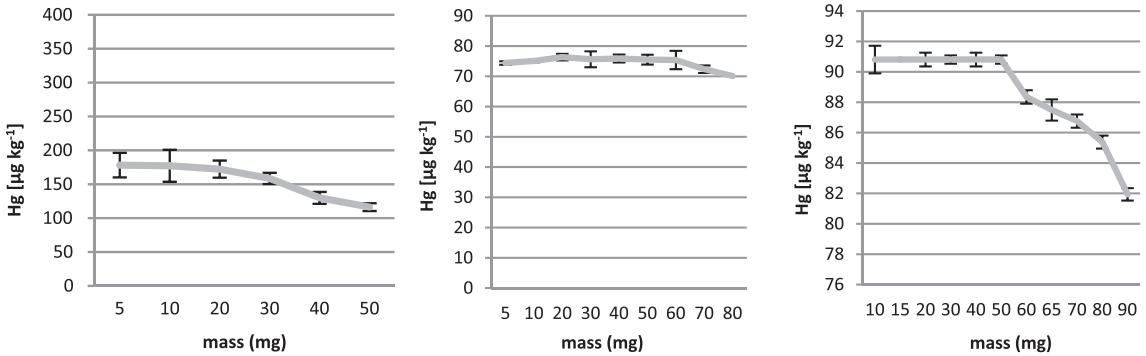

Fig. 1. Study of the effects of different bivalves, fish and crustacean sample masses (mean ± standard deviation) to $n = 10$.

Table 1
Recoveries from the Hg addition and of certified reference materials with precision (expressed as relative standard deviation).

Matrix	Sample value without addition (ng)	After addition of 1 ng Hg		After addition of 10 ng Hg		Precision (%)	Accuracy (%)
		Obtained value (ng)	Recovery (%)	Obtained value (ng)	Recovery (%)		
Fish	0.9	1.8	90	9.4	85	2	91 (DOLT-4)
Bivalve	6.5	7.4	90	15.3	88	4	107 (NIST 1566a)
Crab	3.2	4.2	100	12.1	89	3	103 (MURST-ISS A2)

The lowest and highest concentrations of mercury were obtained for the samples of *B. marinus* and *C. sapidus*, respectively. This can be related to the way each organism metabolizes the mercury furthermore factors such as each organism's mass, size, age, and eating habits (Horvat, Lucotte, & Malm, 2007).

Regarding to the organisms studied in this work, fish take the highest position in the food chain; therefore, the highest concentrations of mercury in fish may be related to the processes of bioaccumulation and biomagnification (Blum et al., 2013; EFSA, 2012). On the other hand, the lower levels of Hg in crustaceans may be a consequence of the sample choices, as the samples were collected during a period of change, in which the crustacean loses its carapace and part of its contamination historic. It is worth mentioning that the sampling in the period of change was motivated by the high consumption of *C. sapidus* in this phase.

Many studies have investigated the concentrations of total mercury in fish, bivalves and crustaceans from different parts of the world (Bisi et al., 2012; Carbonel, Bravo, Fernández, & Tarazona, 2009; Hajeb et al., 2009; Ikem & Egiebor, 2005; McClain, Chumchal, Drenner, & Newland, 2006; Ordiano-Flores, Rosíles-Martínez, & Galván-Magaña, 2012; Schimidt et al., 2015; Zhang, Campbell, & Johnson, 2012). However, this present paper shows the latest determinations of the mercury in fish and shellfish of the TSB, unheard to Salinas da Margarida, and the results demonstrate the need for continued monitoring of element in this region, mainly in predatory fish that exceeded the limits of Brazilian recommendations and were considered inadequate for human consumption.

3.4. Influence of cooking on the mercury concentrations

During cooking, water content of fish tissues decreased on an average by 10–20% for boiled samples, therefore, the humidity values considerated for calculation of wet weight were 67% for fish and 60% for bivalves and crustacean.

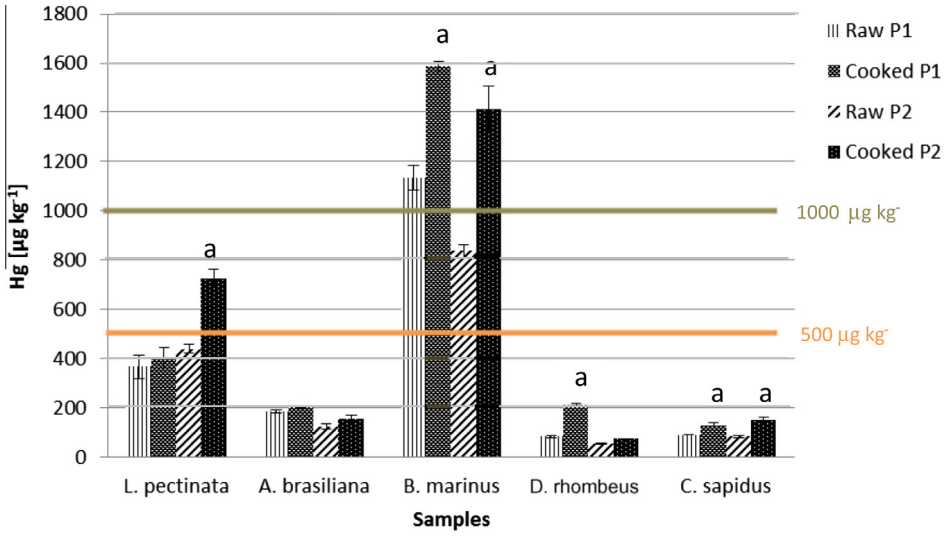

Fig. 2. Total mercury concentrations, in wet weight, with respective deviations shown by error bars for the raw and cooked samples from the 1st and 2nd sampling periods. The small letter "a" represents the results with significant difference ($p < 0.05$), according Student-t test, between raw and cooked samples.

In general, cooked samples presented higher Hg concentration than raw samples (Fig. 2) with significant increase ($p < 0.05$) after cooking. A possible explanation for this result is that the heating can provide the formation of complexes between mercury species and sulfhydryl groups present in tissues, such as methylmercury-cysteine (Clarkson & Magos, 2006). This process can result in a reduction of the possible loss of mercury during the drying step. Another possibility is that the loss of some minerals in the form of salts during cooking may produce the effect of pre-concentrating of the mercury because of an increase in the mercury/mass ratio related to the mass decrease and the maintenance of the initial concentration of mercury. Lastly, the variation of the concentration can be in function of water loss during the cooking, due to the humidity in the cooked samples is lowest than in raw samples, thus, the Hg concentrations are higher in the most of cooked samples (Perelló et al., 2008).

The raw and cooked *B. marinus* samples from P1 and the cooked *B. marinus* samples from P2, besides the cooked *L. pectinata* samples from P2, presented mercury concentrations higher than the values that Brazilian recommendations considers acceptable for human consumption (0.5 mg kg^{-1} for non-carnivorous fish and 1.0 mg kg^{-1} for predator fish). The remaining samples, despite having lower levels than those permitted by current legislation, warn of the necessity of regular monitoring of the food consumed in the region of Salinas da Margarida.

Kalogeropoulos et al. (2012) determined the concentrations of mercury and other elements in raw and cooked samples from 6 species of fish, squid, shrimp and mussels. The authors also showed that the mercury concentrations increased in all samples after cooking. Similar results were found for sardines and tuna by Perelló et al. (2008) and Amyot and Ouédraogo (2011), and according to Perelló et al. (2008) the increase of Hg levels can be due to loss of water and fat.

Although many authors have investigated the mercury concentrations in aquatic organisms around the world, there are still divergences if cooking modifies the results of Hg concentrations. Therefore, the results of previous studies that showed mercury concentrations above of specified limits may be significantly changed by the cooking process, thus, the new concentrations should be considered inadequate for human consumption. This approach can provide more representative data for calculations of the mercury exposure of the population of Salinas da Margarida through the ingestion of marine organisms.

4. Conclusions

This paper presents the latest determinations of mercury in fish and shellfish sampled in the TSB, also taking account the consumption form of seafood by the population. According to our results, the intake of *B. marinus* from Salinas da Margarida could constitute a risk for people, therefore, it is necessary to constantly monitor the mercury in fish and seafood consumed in Salinas da Margarida. It was show that the Hg concentrations can vary significantly after cooking, thus, our results denote that it would be important to evaluate the levels of mercury in food based on the most common way of public consumption.

Acknowledgments

The authors are grateful for the financial support they received from the Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Group of Research in Analytical Chemistry (GPQA) and the Group of Spectrometry, Sample Preparation and Mechanization (GEPAM).

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